EVALUATION OF SOME BIOLOGICAL ACTIVITIES OF TUBER OF *Gloriosa superba* L. (Si-mi-dauk)

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Abstract

Gloriosa superba L. is one of the important medicinal plants. It is used in diseases, like cancer, gout, purgative and so on. In this study, preliminary phytochemical investigation of *G. superba* revealed the presence of alkaloid, flavonoid, glycoside, carbohydrate, starch, α-amino acid, terpenoid, steroid, saponin, tannin, phenolic compound, organic acid and reducing sugar. Cyanogenic glycoside was absent in *G. superba.* Total alkaloids content (1.11 %) of selected plant sample was determined by using gravimetric method of Harbone. The antimicrobial activities of different crude extracts such as pet-ether, ethyl acetate, ethanol, methanol and watery extracts from *G. superba* were determined against six microorganisms such as *Agrobacterium tumefaciens, Bacillus subtilis, Candida albicans, Escherichia coli, Salmonella typhia and staphylococcus aureus* by paper disc diffusion method. According to antimicrobial activity screening, pet-ether extract cannot against all strains of microorganisms. EtOAc and H_2O extracts were observed to possess mild antimicrobial activity whereas EtOH and MeOH extracts have pronounced antimicrobial activity against all tested microorganisms. Antiproliferative activity of EtOH and H_2O extracts of *G. superba* (tuber) against A 549 (lung cancer) and Hela (cervix cancer) was investigated by using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT Assay. Antiproliferative activities of all extracts showed strong activities (IC_{50} <20 µg/ mL against two cancer cell lines.

Keywords: *Gloriosa superba* L. (Si-mi-dauk), phytochemical, total alkaloids, antimicrobial, antiproliferative activities

Introduction

Medicinal plants have been used as sources of medicine in virtually all cultures. In recent years, there have been a gradual revival of interest in the use of medicinal plants in developing countries because herbal medicines have been reported safe and without any adverse side effect especially when compared with synthetic drugs (Parkin *et al*., 2000). *G.superba* is an important medicinal plant belonging to the family Liliaceae which is one of the endangered species among the medicinal plants. (Senthilkumar, 2013) The genus *G. superba* is a native to tropical Africa and is found growing naturally in many countries of tropical Asia including India, Burma, Malaysia and Srilanka. (Nebapure, 2012). It is a semi-woody herbaceous branched climber reaching approximately 5 meters height, with brilliant wavy-edged yellow and red flowers that appears from November to March every year. Fruits are oblong, ellipsoid capsule. Seeds are numerous and rounded. One to four stems arise from a single V-shaped fleshy cylindrical tuber. It has large pharmacological value due to present of an important alkaloid, colchicine, gloriosine and also other biologically active compounds (Jason and Mohamad, 2014). The phytochemical from *G. superba* can act as anti-oxidant and anti-cancer by hormonal action, enzymes stimulators, physical action (contact within the cells) and interference with DNA replication. *G. superba* has the potentiality to inhibit the human carcinoma cell line growth. It is used for the treatment of leprosy, inflammations, skin problems, snake bites, insect bite, gout, asthma, paralysis, cancer, fever and blood disorder (Ravindra *et al*., 2009). The tuberous root stocks of *G. superba* was boiled with sesame oil and was applied twice a day on the joints, affected with arthritis reduces pain. The rural women prefer *G. superba* plant for gynecological disorders like abortion, menstrual trouble, conception disorders, sterility, delivery problems (Simon *et al*., 2016).

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(a) (b) (c)

Figure 1 The photographs of (a) plant of *G. superba*, (b) seed of *G. superba* (c) tuber of *G. superba*

Botanical Aspect of *Gloriosa superba* L.

Family	Liliaceae
Genus	Gloriosa
Species	*superba*
Botanical name	*Gloriosa superba* L.
Myanmar name	si-mi-dauk

Materials and Methods

Collection and Preparation of Selected Medicinal Plant Sample

The tuber of *G. superba* (si-mi-dauk) was collected from Aunglan in Magway Region, during August, 2018. The collected sample was washed with water and air-dried at room temperature. The dried species were made into powder by using grinding machine.

Phytochemical Screening

Preliminary phytochemical tests such as alkaloids, glycosides, carbohydrates, α-amino acids, flavonoids, terpenoids and steroids, saponins, tannins, phenolic compounds, reducing sugars, cyanogenic glycoside and organic acid were carried out according to the appropriate reported methods (Harborne, 1993).

Quantitative Analysis of Total Alkaloids

In tuber of *G. superba*, the total alkaloids content was determined by gravimetric method of Harbone. 5 g of powder sample was weighted into 250 mL conical flask and 200 mL of (20 %) acetic acid in ethanol added, covered and allowed to stand for 4 h and then filtered. The filtrate was concentrated on a water bath at 60 ˚C to one quarter of its original volume and concentrated aqueous ammonium hydroxide solution was added drop-wise to the extract in order to precipitate the alkaloid. The whole solution was centrifuged and the precipitate was collected and washed with 15 % ammonium hydroxide solution. The precipitate obtained as alkaloid was dried in an oven at 60 ˚C for 30 min and weighed. Alkaloid content was calculated and expressed as a percentage of the weight of sample analyzed. The percentage of total alkaloid content was calculated as:

Percentage of total alkaloids (%) = Weight of residue×100 / Weight of sample taken

(Harborne., 1993)

Preparation of Various Crude Extracts

Dried powdered tuber sample of *G. superba* (25 g) was extracted with ethanol 50 mL, for 3 days at room temperature (3 times). The mixture was filtered with filter paper. The filtrate was concentrated by removal of the solvent to give the ethanol extract. Similarly, pet-ether, ethanol, methanol and watery extracts were prepared the above procedure.

Watery extract of dry powder sample (25 g) was prepared by boiling with 50 mL of distilled water for 6 h and filtered. The filtrate was concentrated by removal of the water to give the watery extract.

Screening of Antimicrobial Activity of Crude Extracts of the *G. superba* L.Tuber

The screening of antimicrobial activity of the various crude extracts such as PE, EtOAc, EtOH, MeOH and H_2O extracts of the tuber of *G. superba* were carried out by paper disc diffusion method at Department of Botany, University of Yangon. Six microorganisms namely *Agrobacterium tumefaciens, Bacillus subtilis, Candida albicans, Escherichia coli, Salmonella typhi,* and *Staphylococcus aureus* were used for this test. 10 µL of each crude extract was put on paper disc and air-dried at room temperature for 24 h. The test organisms were incubated in test broth medium containing glucose (0.5 g), polypeptone (0.2 g) and distilled water (100 mL) at appropriate temperature for 24 h. Assay medium containing glucose (1.0 g), polypeptone (0.2 g), agar (1.6 g) and distilled water (100 mL) were placed in beaker and the contents were heated for 10 min. The assay medium was put into sterilized conical flask and plugged with cotton wool and then autoclaved at 121 ˚C for 15 min. After cooling down to 40 ˚C, 0.1 mL of suspended strain was inoculated to the assay medium with the help of a sterilized disposable pipette near the burner. About 20 mL of medium was poured into the sterilized petri-dishes and allowed to set the medium. The dishes were cooled for 2 h at room temperature. After solidification, paper discs impregnated with samples (crude extracts) were applied on the ager plates and incubated at 27 ˚C for 24-36 h. Clear zones (inhibition zones) surrounding the paper discs indicate the presence of bioactive metabolites which inhibit the growth of test organisms. The diameter of clear zone around the well were measured with digital calipers in millimeter. The antimicrobial activity was determined by measuring the clear zone around the wells.

Determination of Antiproliferative Activities of Ethanol and Watery Extracts of the *G. superba* by using MTT assay

In in *vitro* antiproliferative activities of EtOH and H_2O extracts of the tuber of *G. superba* were determined against two human cancer cell lines such as A 549 (lung cancer) and Hela (cervix cancer) (Win *et al*., 2015). These tests were done at Department of Natural Products Chemistry, Institute of Natural Medicine, University of Toyama, Japan.

Preparation of medium for cell growth

500 mL of minimum Essential Medium (α MEM) was added to 50 mL of fetal bovine serum (FBS) solution to prepare supplemented medium for all cell types.

From the above medium solution, 200 mL of this supplemented medium was taken and mixed with 2 mL of Non-Essential Amino Acid (NEAA) that was used for A549 cancer cell line.

From the above medium solution, only 100 mL of these supplemented medium was also used for Hela cell line.

Preparation of phosphate buffer saline solution

The phosphate buffer saline (PBS) powder (4.8 g) was dissolved in 500 mL of ultra-pure water sterilized and kept in the refrigerator.

Preparation of cell growth

The cell was taken from the stock and transferred into a 15 mL centrifuge tube followed by addition of respective medium (5 mL). The suspension cell was centrifuged in the refrigerated centrifuge machine (1000 rpm) for 3 min. After the centrifugation, the supernatant was carefully removed without disturbing the cell pellet. And then fresh medium (2 mL) was gently added to the side of the tube and slowly pipetted up and down 2 to 3 times to re-suspend the cell pellet. The suspension cell was centrifuged for 3 min. The supernatant was carefully removes without disturbing the cell pellet. The cell suspension was diluted with 6 mL of medium. Finally, the cells were transferred to the desired sterile container and the cells were incubated until 70-100 % cell confluences for 7 days at an incubator.

Preparation of sample and control solutions by Serial Dilution Method

1 mL of each of the EtOH and H_2O extracts of the tuber of *G. superba* was dissolved in each of 100 µL of DMSO solution to get 10000 µg/mL sample solution. The solution was necessary to vibrate at vibrator. The two eppendroff tubes for each sample were used for serial dilution. The fresh medium (686 µL) was added in the first eppendroff tube and then another fresh medium (540 µL) was put into the second tube. 14 µL of stock sample solution was added to the 686 µL fresh medium with first eppendorff tube and vibrated well for solubility. And then 60 µL from the first eppendorff tube was added to the 540 µL fresh medium with second eppendorff tube and slowly pipetted up and down 2 to 3 times. Finally, 200 and 20 µg/mL of serial solution were obtained and kept in the refrigerator.

The control solutions were serially prepared as described above procedure. Instead of sample extract, 5-fluorouracil (5FU) was used for the positive control. Only DMSO was used for negative control.

Procedure for screening of antiproliferative activity by MTT Assay

After the cell growth, the 70-100 % cell in the medium was aspirated with aspirator. Cell was washed with PBS (5 mL) for two times. The cell was trysinased with trypsin

(4 mL) and incubated for 2-3 min. And then the medium (1 mL) was added to stop trypsinization. The cell suspension was transferred to 15 mL centrifuge tube. The tube (cell suspension) was centrifuged in the refrigerated centrifuge machine (2000 rpm) with the same centrifuge tube for 3 min. After the centrifugation, the supernatant was carefully removed without disturbing the cell pellet and the cell was found at the bottom of the centrifuge tube. The cell in the centrifuge tube was added with fresh medium (3 mL) gently to the side of the tube and slowly pipetted up and down 2 to 3 times to re-suspend the cell pellet. The number of cell was counted with Haemocytometer.

The solution (10 mL) was mixed with the tryphan blue (40 mL) in the chamber and the covered slip was cleaned with alcohol (70% EtOH). The chamber was dried and the cover slip was fixed in position. The cell was harvested and the (10 µL) of the cells added to the Haemocytometer (do not overfill). And then the chamber was placed in the inverted microscope under a 10 X objective and phase contrast was used to distinguish the cell. The cell was counted in the large, central gridded square (1 mm). The gridded square was circled and multiplied by 10^4 to estimate the number of cell per millimeter. The number of cell was counted by the following equation.

No. of cell in stock = counted cell/ 4×10^4 ×dilution factor × volume of stock cell solution

After the cell counting, the medium was added with (120 mL/ 120×103 µL) of medium for 12 plates. 100 µL medium of cell was filled in 96 well plates. The cell in 96 well plates was incubated in an incubator for 24 h.

After incubation, the medium was removed by absorption machine (very carefully) and washed with 100 µL of the different concentrations of sample and control solution was added in the 96 well plates. The sample solutions in 96 well plates with cell was incubated in an incubator for 72 h.

The sample solution with cell and medium was added with 100 µL MTT reagent. And then the 96 well plates were incubated in an incubator for 3 h. After incubation, the absorbance of each solution was measured at 450 nm by using UV-visible spectrophotometer for Hela cell line and for A 549 cell line, 96 well plates were added 100 µL DMSO solution and then measured the absorbance at 570 nm by using UV-visible spectrophotometer. The percent cell viability activity was calculated by the following equation.

% Cell viability = [(Abs (test sample) – Abs (blank)/ (Abs (control)-Abs (blank))] ×100

Where,

Abs (test sample) = absorbance of test sample solution

Abs (control) = absorbance of DMSO solution

Abs (blank) = absorbance of MTT reagent

IC_{50} (50 % inhibition concentration) values were calculated by linear regressive excel program. The standard deviation was also calculated by the following equation.

$$\text{Standard Deviation } (SD) = \sqrt{\frac{(\bar{x}\text{-}x_1)^2 + (\bar{x}\text{-}x_2)^2 + ...(\bar{x}\text{-}x_n)^2}{(n\text{-}1)}}$$

Where,

$\bar{X}$ = average % inhibition

$X_1, X_2, \ldots\ldots, X_n$ = % cell inhibition of test sample solution

n = number of times

Results and Discussion

Types of Phytochemicals Present in *G. superba* L.

In order to find out the types of phytochemical constituents present in the tuber of *G. superba*, the phytochemical tests were preliminary carried out according to the reported procedure. From the data findings, it was observed that various secondary metabolites such as alkaloid, flavonoid, glycoside, carbohydrate, starch, α-amino acid, terpenoid, steroid, saponin, tannin, phenolic compound, organic acid and reducing sugar were present, however cyanogenic glycoside was not detected in the sample (Table 1). According to the results, it can be seen that tuber of *G. superba* might contain potent bioactive secondary metabolites.

These secondary metabolites contribute significantly towards the biological activities of medicinal plants such as antimicrobial and antiproliferative activities.

Table 1 Phytochemicals Present of *G.superba* L.

No	Types of compounds	Extract	Test reagents	Observation	*G. superba*
1	Alkaloid	1 % HCl	Mayer's reagent	ppt(white)	+
			Dragendorff's reagent	ppt(orange)	+
			Wagner's reagent	ppt(reddish brown)	+
2	Glycoside	H_2O	10 % lead acetate	ppt (white)	+
3	Carbohydrate	H_2O	10 % α-naphthol, H_2SO_4	red ring	+
4	α-amino acid	H_2O	Ninhydrin reagent	purple color	+
5	Flavonoid	70 % EtOH	Mg turning and conc: HCl	pink color	+
6	Terpenoids	$CHCl_3$	Acetic anhydride and conc: H_2SO_4	pink color	+
7	Steroid	PE	Acetic anhydride and conc: H_2SO_4	green color	+
8	Tannin	H_2O	0.1 % $FeCl_3$	brownish green	+
9	Phenolic compound	H_2O	5% Ferric chloride	dark blue	+
10	Saponins	H_2O	Distilled water	frothing	+
11	Reducing Sugars	H_2SO_4(dil)	Benedict's solution	brick red ppt	+
12	Organic acid	H_2O	Bromocresol green	yellow color	+
13	Starch	H_2O	Iodine solution	bluish black ppt	+
14	Cyanogenic glycoside	H_2O	H_2SO_4, sodium picrate	No brick red	–

(+) present, (-) absent, ppt = precipitate.

Quantitative Analysis of Total Alkaloids

In this study, the total alkaloid content of tuber of *G. superba* was estimated by Harbone method. According to this method, the total alkaloids content is found to be 1.11 % in selected sample. Alkaloid acts as a local anaesthetics, anti-tumor, anti-malarials, anti-bacterials agents and pain killer. The higher the total alkaloid contents, the more activity against the various diseases (Megale *et al.*, 2013).

Antimicrobial Activities of Different Crude Extracts

Four crude extracts such as EtOAc, EtOH, MeOH and H_2O extracts of the tuber of *G. superba* were subjected to screening of antimicrobial activity against six different pathogenic microbes such as *Agrobacterium tumefaciens, Bacillus subtilis, Candida albicans, Escherichia coli, Salmonella typhi,* and *Staphylococcus aureus* using ager disc diffusion method. This method is based on zone diameter including the disc diameter, in millimeter (mm). The larger the zone diameter, the higher the activity is. According to the results, PE extract was not against all tested microorganisms because the active constituents present in selected sample, it may not dissolve in non-polar solvent medium. EtOAc extract showed active against (16 – 30 mm) in *A. tumefaciens,*

B. subtilis, *C. albicans*, *E. coli* but does not show in *S. typhi* and *S. aureus*. H_2O extract can inhibit five types of microbes (14 - 28 mm) except *B. subtilis*. Whereas EtOH and MeOH extracts were found to be high with diameter zones of inhibition ranged from 12 to 30 mm against all tested microorganisms as shown in Table 2. Among the extracts, EtOH and MeOH extracts can against six different pathogenic microbes. Therefore these extracts showed higher activity than other extracts, as shown in Figure 3.

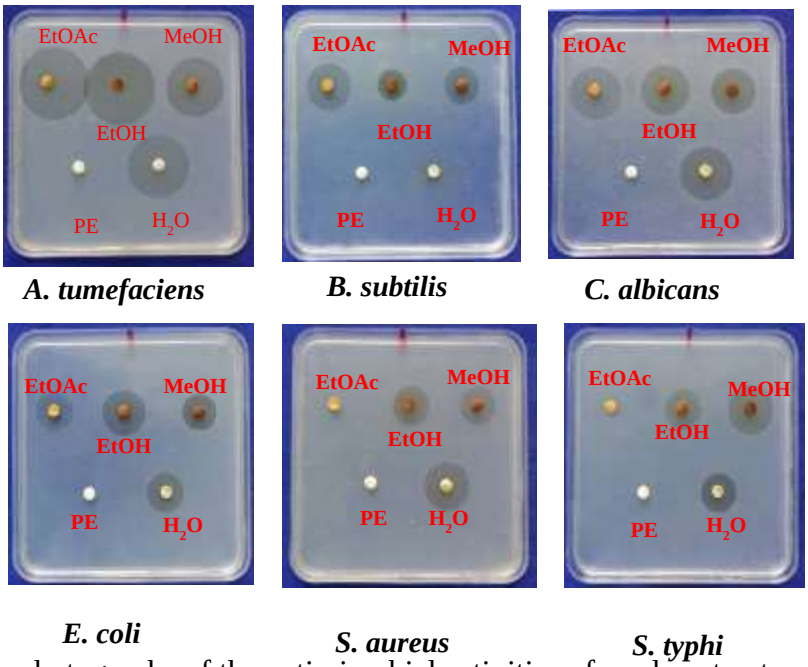

Figure 2 The photographs of the antimicrobial activities of crude extracts of *G. superba* L.

Table 2 Antimicrobial Activities of Crude Extracts of *G. superba* L.

No.	Microorganisms	PE	Inhibition zones diameters(mm) of different crude extracts			
			EtOAc	EtOH	MeOH	H_2O
1	*A. tumefaciens*	-	30 (+++)	30 (+++)	26 (+++)	28 (+++)
2	*B. subtilis*	-	18 (+++)	12 (+)	16 (++)	-
3	*C. albicans*	-	30 (+++)	28 (+++)	26 (+++)	26 (+++)
4	*E. coli*	-	16 (++)	22 (+++)	16 (++)	16 (++)
5	*S. typhi*	-	-	14 (++)	18 (+++)	14 (++)
6	*S. aureus*	-	-	14 (++)	14 (++)	16 (++)

Disc size-6 mm

10-12 mm(+) =weak activity, 13-17(++) = high activity, > 18 mm(+++) = very high activity

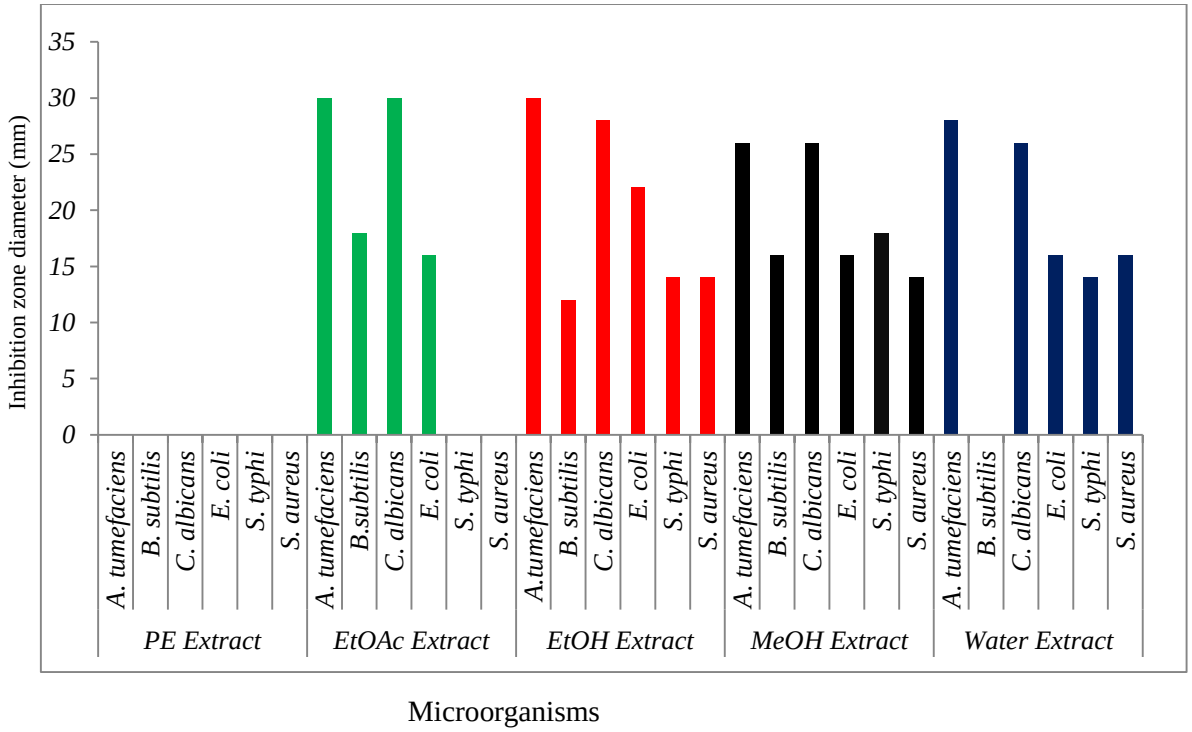

Figure 3 A bar graph of antimicrobial crude extracts *G. superba* L.

Antiproliferative Activity of the EtOH and H_2O Extracts of the *G. superba* L.

The antiproliferative activity is the activity relating to a substance used to prevent or retard the spread of cells, especially malignant cells, into surrounding tissues. Antiproliferative activity of the sample was studied *in vitro* against human cancer cell lines. Screening of antiproliferative activities of ethanol and watery extracts from the tuber of *G. superba* was done against two human cancer cell lines such as A 549 (human lung cancer) and Hela (human cervix cancer). Antiproliferative activity was expressed as the IC_{50} (50 % inhibitory concentration) value. 5-Fluorouracil was used as positive control. The antiproliferative activity of ethanol and watery extracts are summarized in table 3.

Since the lower the IC_{50} values, the higher the antiproliferative activity. For both Hela and A 549 cell lines, the EtOH and H_2O extracts of the tuber of *G. superba* showed potent activities (IC_{50} <20 µg/ml). These results indicated that the tuber of *G. superba* could be regarded as an anti-cancer herbal medicine as well as a potential crude drug source for the development of anti-cancer compounds.

Table 3 Antiproliferative Activities of Crude Extracts of *G. superba* Against Two Types of Cancer Cell Lines

Extracts	Hela (cell viability)			A 549 (cell viability)		
	20 µg/mL	200 µg/mL	IC 50 µg/mL	20 µg/mL	200 µg/mL	IC 50 µg/mL
EtOH	8.00	21.45	<20	11.62	25.35	<20
H_2O	8.43	13.18	<20	14.73	17.39	<20

Hela = human cervix cancer
A 549 = human lung cancer

Table 4 Antiproliferative Activities of Positive Control (5FU) Against Two Types of Cancer Cell Lines

Cell lines	Concentrations (μM)	% Cell viability	IC_{50} (μM)
Hela	2	91.44	
	10	85.22	15.84
	20	24.39	
A549	2	136.24	
	10	70.45	19.06
	20	47.89	

Conclusion

From the overall assessment concerning with the investigation of phytochemicals and biological activities on the tuber of *G. superba* L., the following inferences can be deduced. Various types of secondary metabolites such as alkaloid, flavonoid, glycoside, carbohydrate, starch, α-amino acid, terpenoid, steroid, saponin, tannin, phenolic compound, organic acid and reducing sugar were present, however cyanogenic glycoside was not detected in the sample. Total alkaloids content of the tuber of *G. superba* is 1.11 %. According to the screening of antimicrobial activity, PE extract was not against all tested microorganisms because the active constituents present in selected sample, it may not dissolve in non-polar solvent medium. EtOAc extract showed highly against (16 – 30 mm) in *A. tumefaciens, B. subtilis, C. albicans, E. coli* but does not showed in *S. typhi and S. aureus.* H_2O extract can inhibit five types of microbes (14 - 28 mm) except *B. subtilis.* Whereas EtOH and MeOH extracts were found to be high with the zones of inhibition ranged from 12 to 30 mm against all tested microorganisms. Among the extracts, EtOH and MeOH extracts can inhibit against six different pathogenic microbes. Therefore, these extracts showed higher activity than other extracts. Moreover, the EtOH and H_2O extracts of the tuber of *G. superba* showed hightly antiproliferative activities (IC_{50} <20 μg/ml). Therefore, selected sample can prevent human cervix cancer (Hela) and human lung cancer (A 549). With these results, the tested plant might be useful for the treatment of bacteria and fungus infected diseases and be used as anticancer agent.

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References

Harborne, J. B. (1993). Phytochemistry Dictionary: A Hand Book of Bioactive Compounds from Plants, London.

Jason, R. J. and Mohamed, I. M. (2014). "Quantification of Colchicine in Various Parts of *Gloriosa superba* by HPLC". *J. Chem and Pharm. Sci.,* vol. 2, pp. 53-55

Megale, S. and Elango, R. (2013). "Antifungal Activities of Tubers and Seeds Extracts of *Gloriosa superba* L. Against in Human Pathogens". *Int. J. Sci. Res.,* vol. 2, pp. 12-24

Nebapure, S. M. (2012). "Extraction and bioefficacy of *Gloriosa superba* L. Against *Spodoptera litura* (FABRICUS) and *Tribolium castaneum* (HERBST.)" *Division of Entomology India*. *Agri. Res. Inst.*, New Delhi.

Parkin, D. M., Bray, F., Ferlay, J. and Pisani, P. (2000). The World Cancer: Globocan 2000". *Int. J. Cancer*., vol. 94, pp. 153-156

Ravindra, A. and Mahendra, K. R. (2009). "Review: Current Advances in *Gloroisa superba* L". *J. Biol. Diversity*, vol. 10 (4), pp. 210-214

Senthilkumar, M. (2013). "Phytochemical Screening and Antibacterial Activity of *Gloriosa superba* L." *Int. J. Pharm. and Phytochem. Res.,* vol. 5(1), pp. 31-36

Simon, S. E. and Jayakumar, F. A. (2016). "Anti-oxidant Activity and Anticancer Study on Phytochemicals Extract from Tubers of *Gloriosa superba* against Human Cancer Cell (Hep-G2)". *J. Pharm. And Phytochem.,* vol. 3, pp. *2332-2347*

Win, N. N., Ito, T., Aimaiti, S., Imagawa, H., Ngwe, H., Abe, I. and Morita, H. (2015). "Kaempulchraols A-H, Diterpenoids from the Rhizomes of *Kaempferia pulchra* collected in Myanmar". *J. Nat. Prod*., vol. 78, pp. 1113-1118